Present and Future of Pathogen Reduction for Red Cells and Whole Blood

24 de Junio de 2016

Dr. Markus M. Müller, M.D.

Assistant Medical Director

Department Head Blood Donation

Institute for Transfusion Medicine and Immohematology

German Red Cross Blood Transfusion Service

Baden-Württemberg- Hessen

University Clinics of the Goethe University Frankfurt/Main, Germany

Dr. Anaïs ALTMEYER, Ph.D.

Transfusion Swiss Red Cross

Dr. Veronika Brixner, M.D.

Dr. Christof Geisen, M.D.

Dr. Soraya Amar El Dusouqui , M.D.

Prof. Dr. Michael Schmidt, M.D.

Prof. Dr. Erhard Seifried , M.D..

Institute for Transfusion Medicine and Immunohematology
University Clinics of the Goethe University Frankfurt/Main
GRC Blood Transfusion Service Baden-Württemberg - Hessen

UNIVERSITÄTS KLINIKUM FRANKFURT
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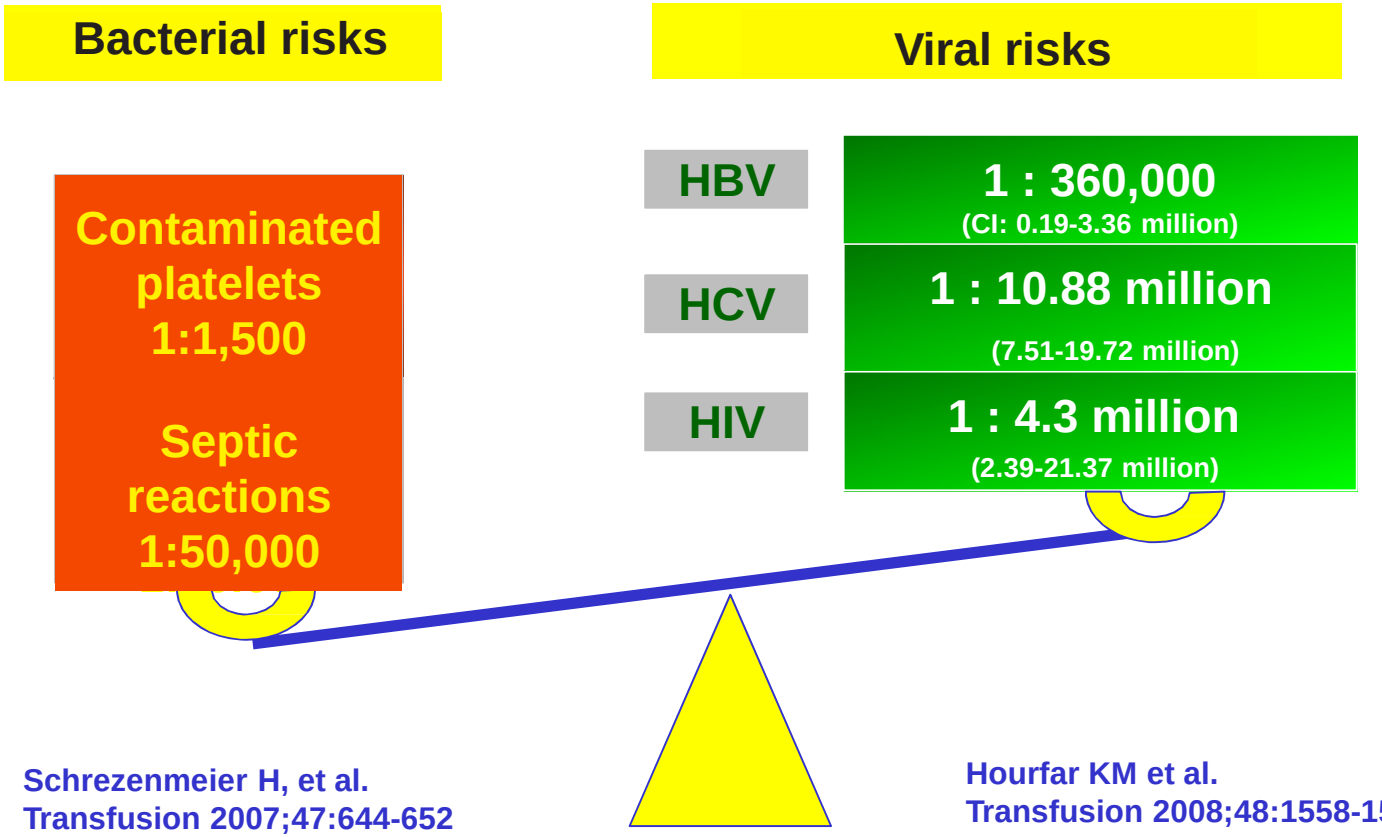
Disclosures

- Novartis Oncology Inc., New Jersey, USA: honorarium for consultation report
- Fresenius KABI GmbH, Bad Homburg, Germany: honorarium for lectures (satellite symposia)
- CERUS Inc., CA, USA & CERUS Europe BV, The Netherlands: support for research projects/research grant & honorarium for lectures (satellite symposium)

Residual risk for viral and bacterial transmission via transfusions



Schrezenmeier H, et al.
Transfusion 2007;47:644-652

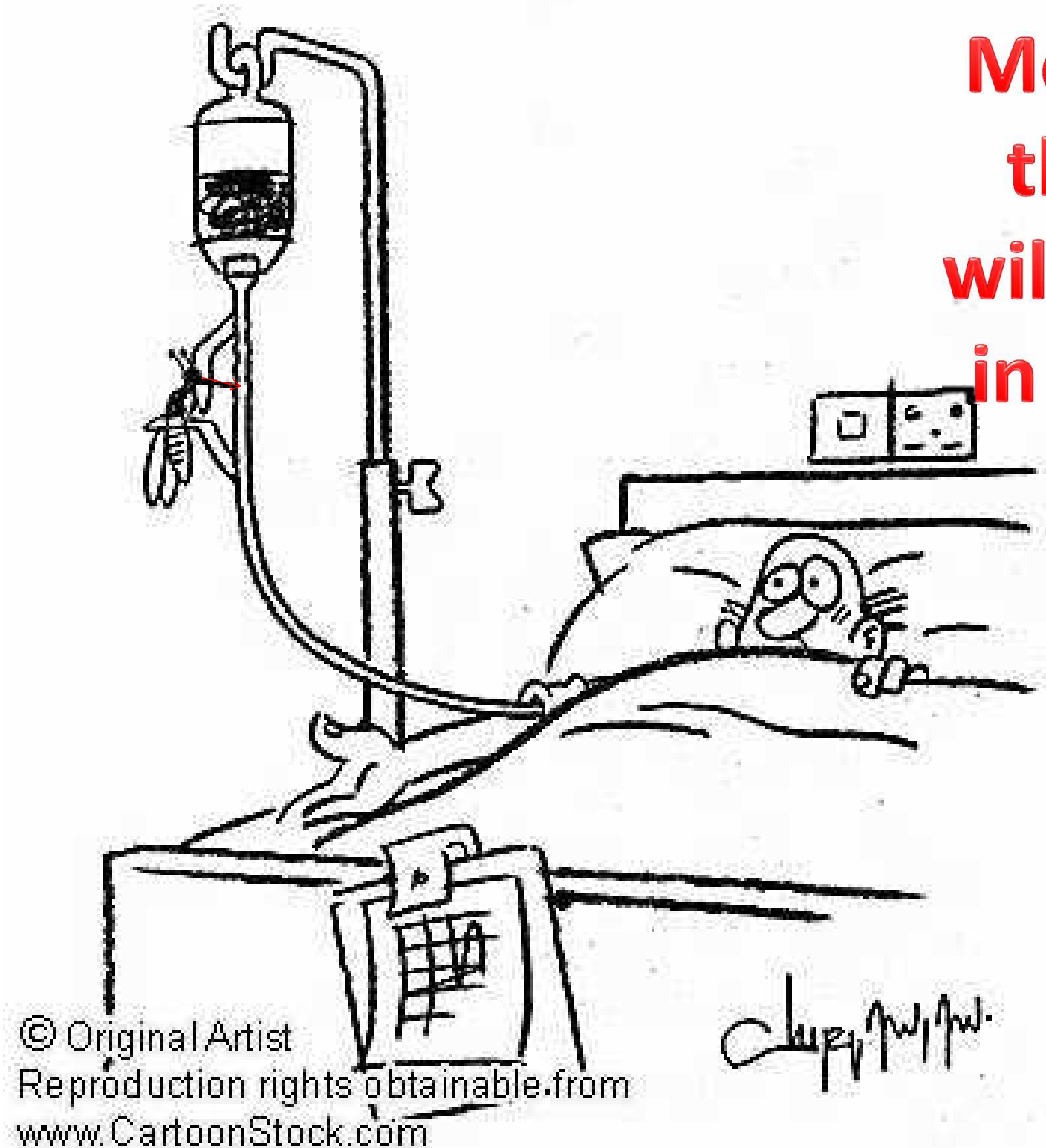
Hourfar KM et al.
Transfusion 2008;48:1558-1566

re-emerging pathogens

Year	Pathogen
1981/´82	HTLV III (= HIV-1) / AIDS
1986	HIV-2
1988	Hepatitis E (Caliciviridae)
1989	Hepatitis C (Flaviviridae)
1992	Vibrio O 139
1992	Bartonella hensellae
1993	Sin Nombre Virus
1995	Hepatitis G (Flaviviridae)

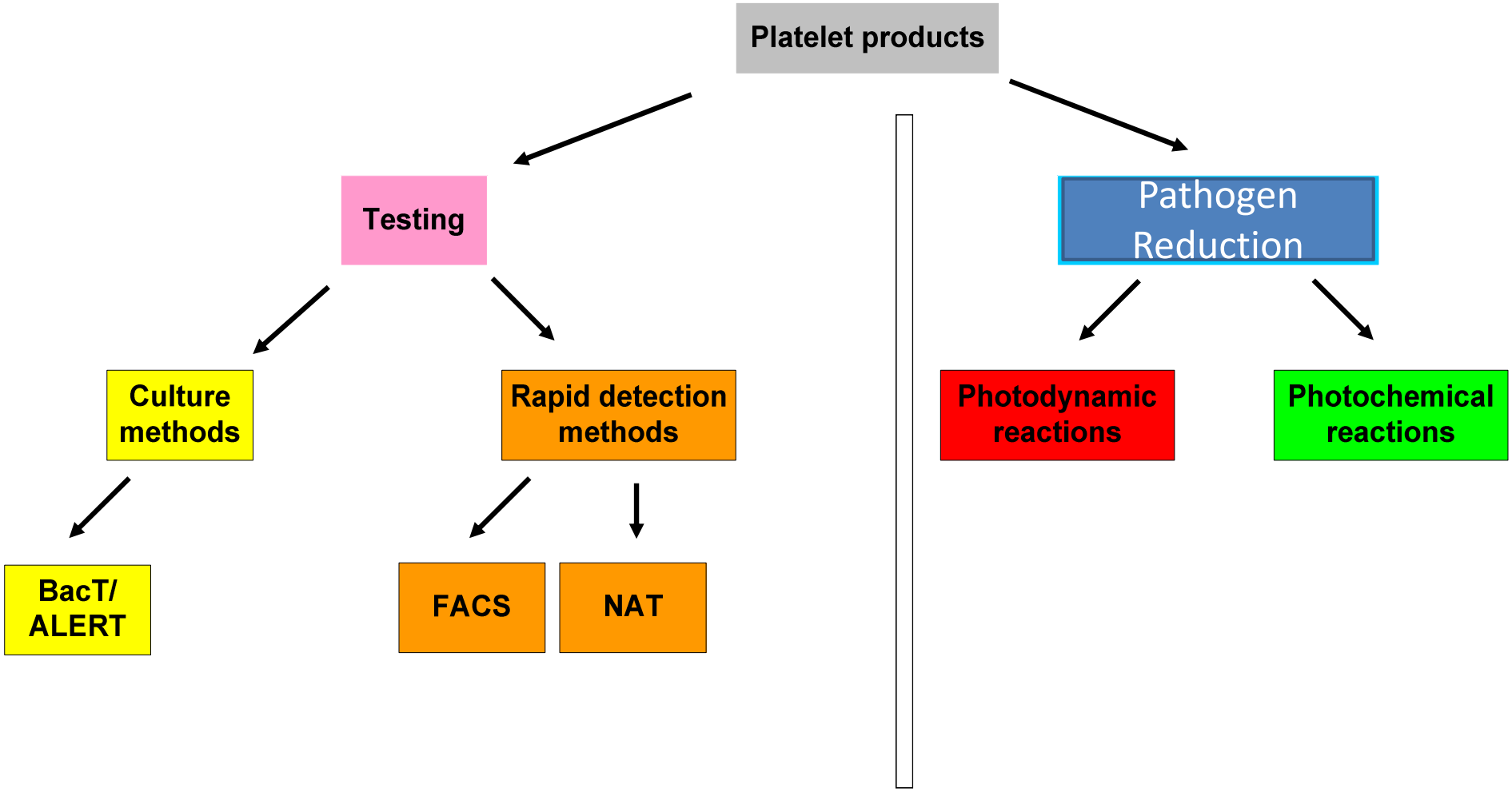
Year	Pathogen
1995	HHV 8[1]
1996	Variant CJD (vCJD) / Prions
1997	Avian Influenza Virus A (H5N1)
1999	West Nile Virus (WNV; Flaviviridae) in USA
2003	SARS (Coronaviridae)
2003	Monkeypox Virus
2004	Metapneumo Virus
2005	Chikungunya Virus

During the last decade in Europe: Malaria, Dengue Virus, Influenza, Swine Flu, Avian Influenza, West Nile Virus, Chikungunya Virus, Hepatitis E Virus, Q Fever, Babesia, Zika Virus, etc. ….



Most probably, the situation will not improve in the future ...

Possible Risk Reduction Strategies
Example: Platelet Concentrates



Testing = Reactive Strategy
Pathogen Reduction = Proactive Strategy

Testing

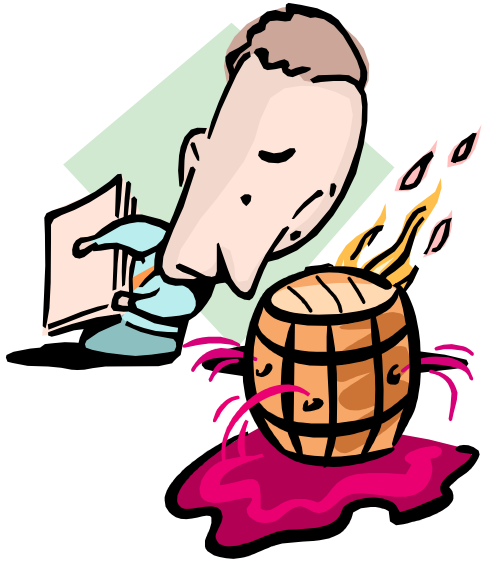
- What is the next bug?

- Great achievements, but outdated already?

&

Pathogen Reduction:

- Where are the gaps?

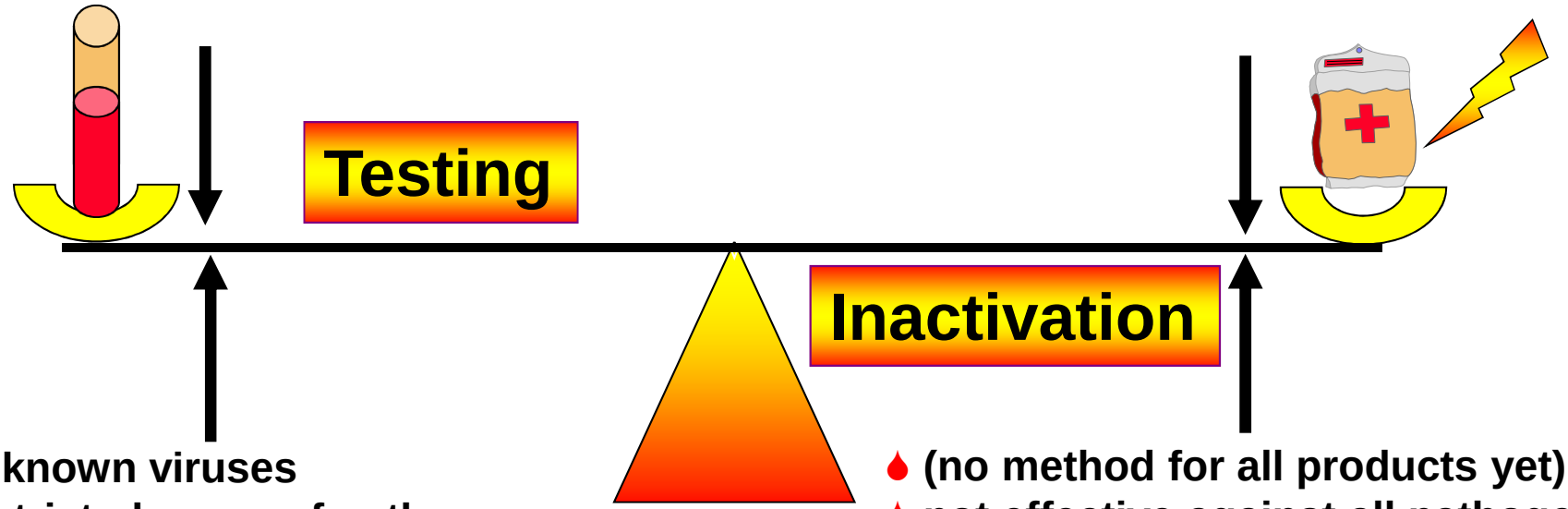


- Not all germs
- not for all blood products by one methodology

Reducing the Risks: Testing versus Inactivation

- all products
- no additives

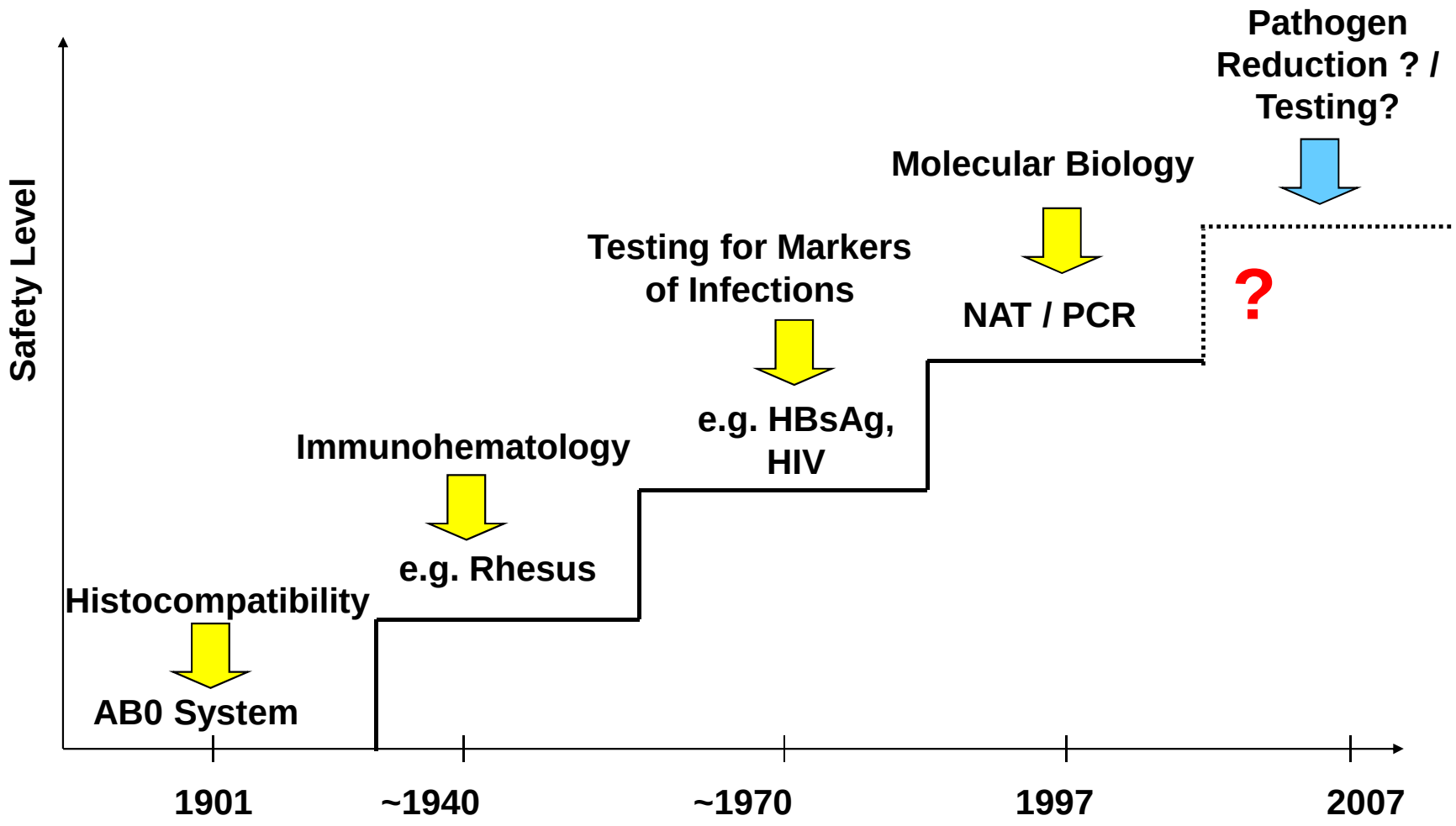
- unknown viruses
- leukocytes
- broad range of pathogens



- unknown viruses
- restricted range of pathogens
- delay in product release
- costs dependent on number of pathogens tested for

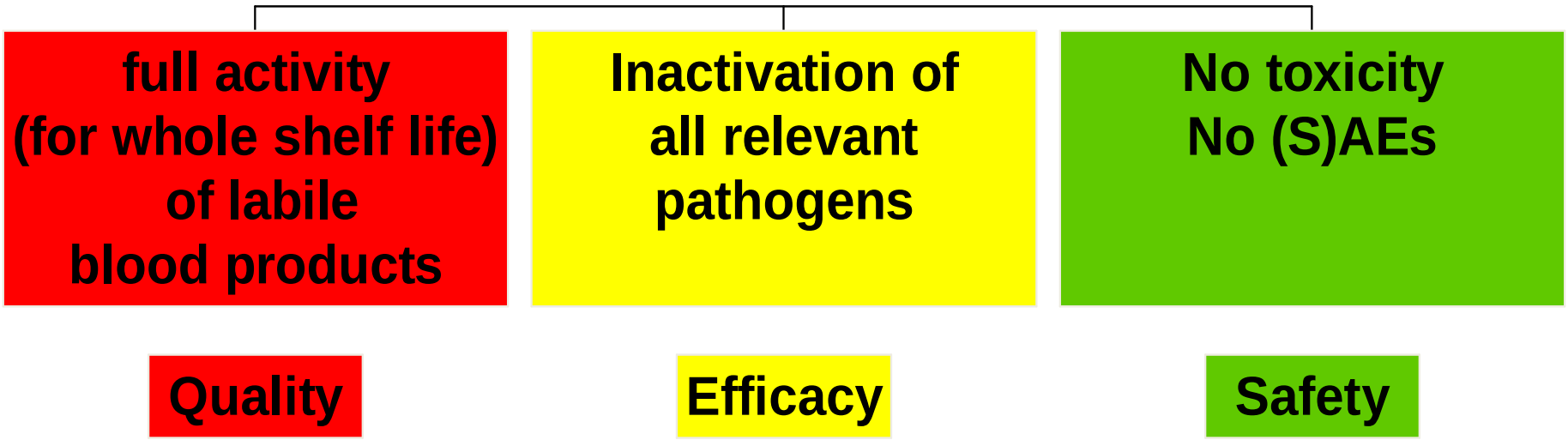
- (no method for all products yet)
- not effective against all pathogens (e.g. spec.viruses (only some methods); prions; bacterial spores)
- loss of activity dependent on methodology
- increase in production costs

Milestones in the Development of Transfusion Safety



Adapted from Klueter 2004

Specifications for Pathogen Inactivation Methods



Problems to be solved:

- **Safety of Production and Clinical Use ?**
  - **Especially in risk populations (pregnancy; intrauterine**
  - **transfusion, pre-term immunocompromised babies, etc.) ?**
- **Feasibility? Integration in production processes ?**
- **Costs ! Might these become prohibitive ?**

Phase 3 Evaluation of INTERCEPT RBC: In vitro characterisation and patient outcome

A Randomized Controlled Double-Blind Phase 3 Study to Assess Characteristics of S-303 Treated RBC Components and Evaluate Safety and Efficacy in Patients Requiring Transfusion Support for Acute Anemia

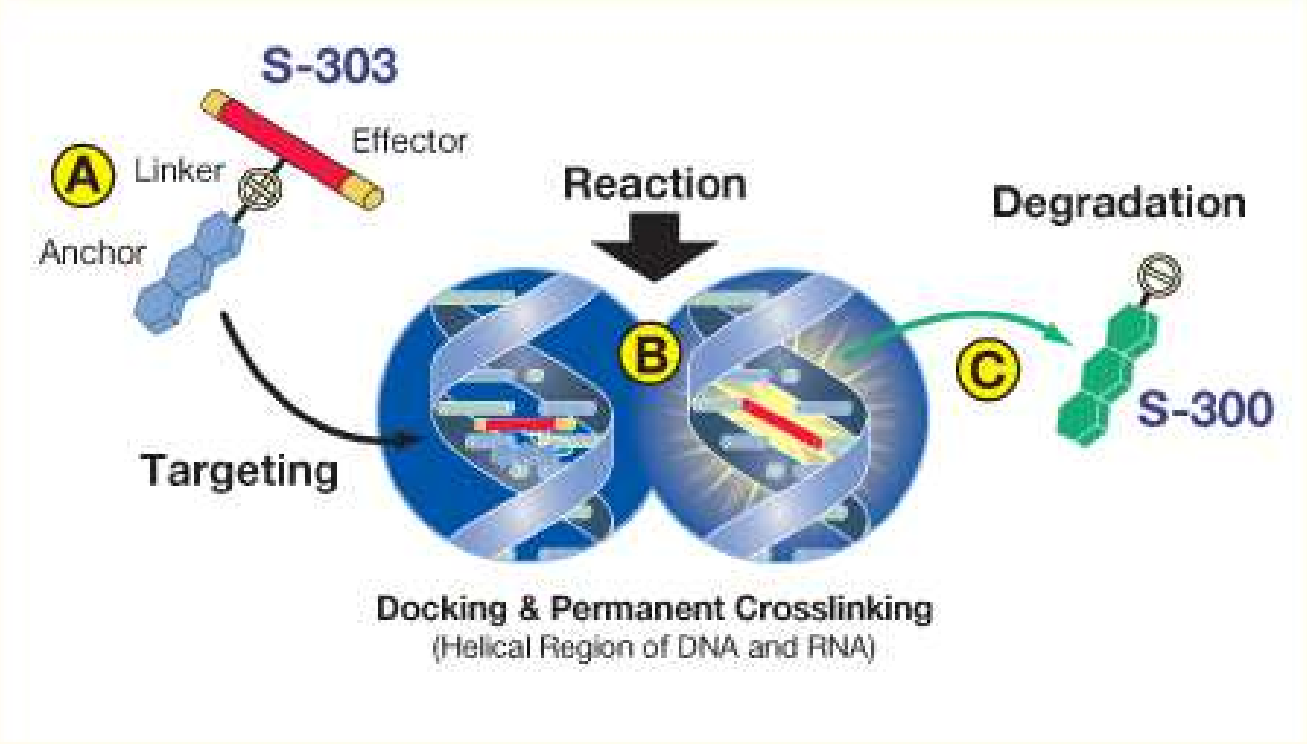
Dr. Markus M. Mueller, M.D.

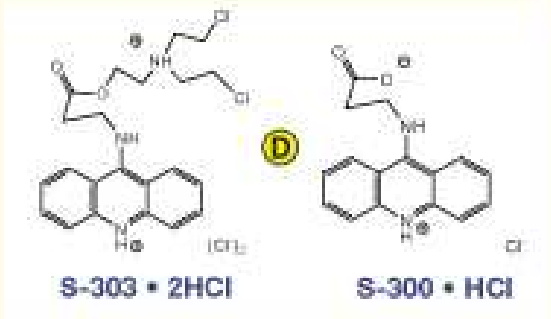
German Red Cross Blood Transfusion Service

Frankfurt/Main, Germany

S-303 Mechanism of Action

Figure 1: S-303 Treatment Process Mechanism of Action





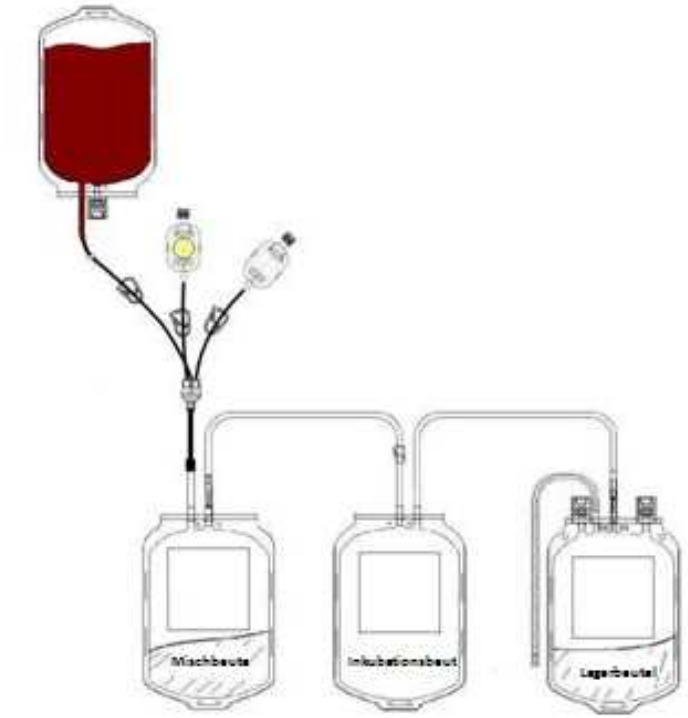
- Anchor selectively targets nucleic acids
- Effector crosslinks nucleic acids
- Linker temporarily joins anchor and effector
- Cross-linking reaction is faster than linker degradation
- Degradation yields unreactive by-products

S-303 is a nucleic acid-targeted alkylator that quickly diffuses into viruses, bacteria, parasites and blood cells and is designed to react quickly and decompose
Glutathione (GSH) is used to quench side reactions of the effector with other biological materials

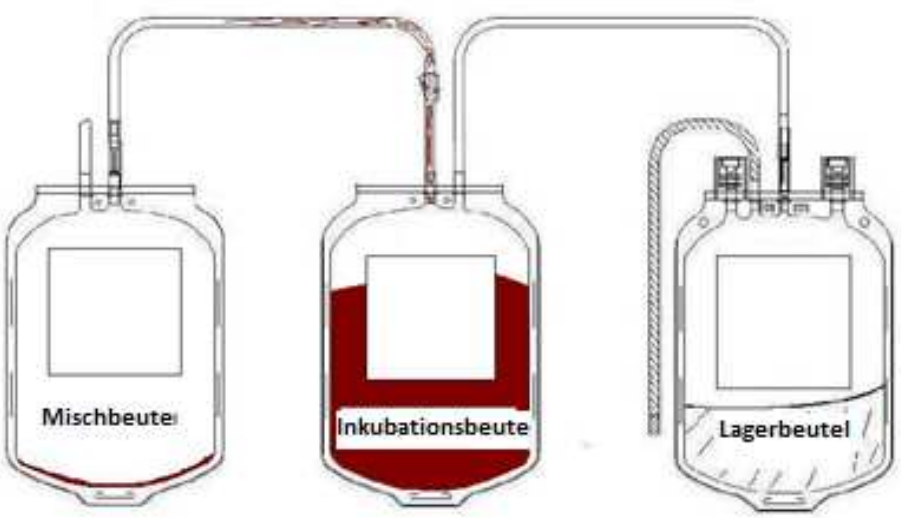
Study design

- Randomized, controlled, mulitcenter Phase 3 clinical trial
- Population: 50 transfused elective cardiovascular surgery patients under going first time CABG or valve repair or combination
- Intervention: Transfusion of S-303 treated RBC, stored up to 35 days in SAG-M, administered according to local standard clinical practice
- Comparator: Conventional RBC stored up to 35 days in SAG-M, administered according to local standard clinical practice. To prevent unblinding, the conventional RBC is transferred into the storage bag of the pathogen inactivation system
- Outcome:
  Primary endpoint: S-303 RBCs are non-inferior to conventional RBC with respect to grams of Hb per RBC unit
  Secondary endpoint:
  a) Incidence of renal insufficiency as measured by creatinine
  b) Incidence of hepatic insufficiency as measured by total bilirubin
  full safety assessment
- Timeframe: RBC support up to 7-days (day of surgery plus 6 days post-op)

RBC, Glutathione and S-303 are mixed

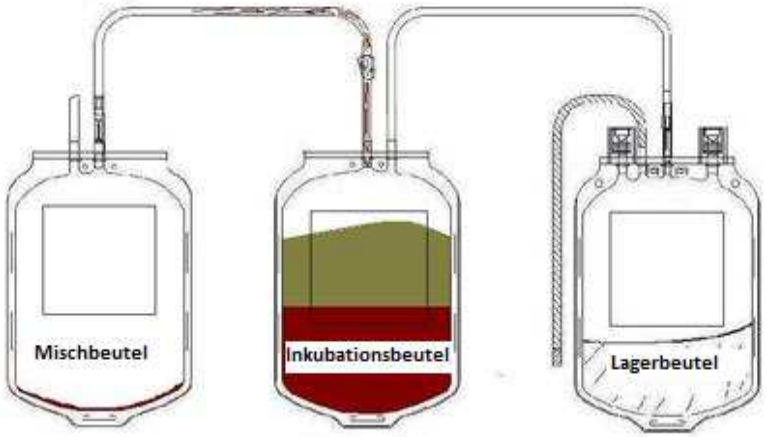


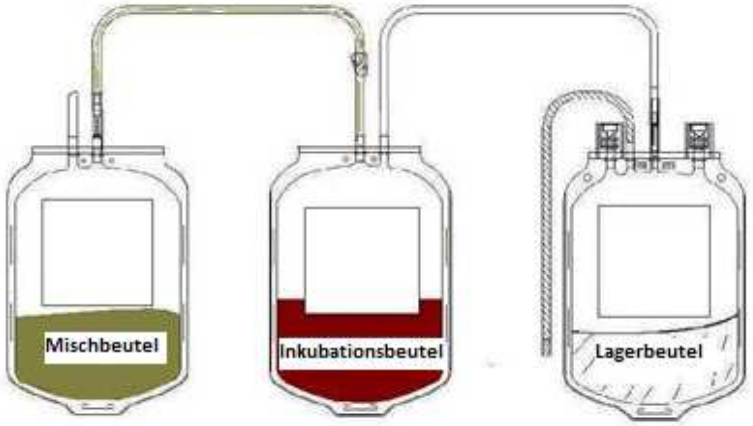
Incubation



Incubation at 20°C – 25°C for 18-24h

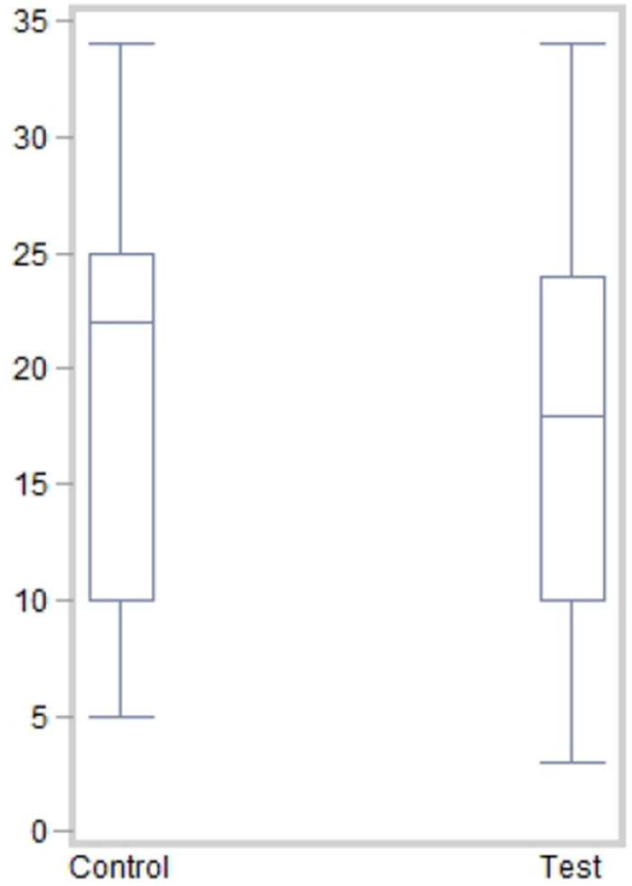
Removal of S-303 degradation products





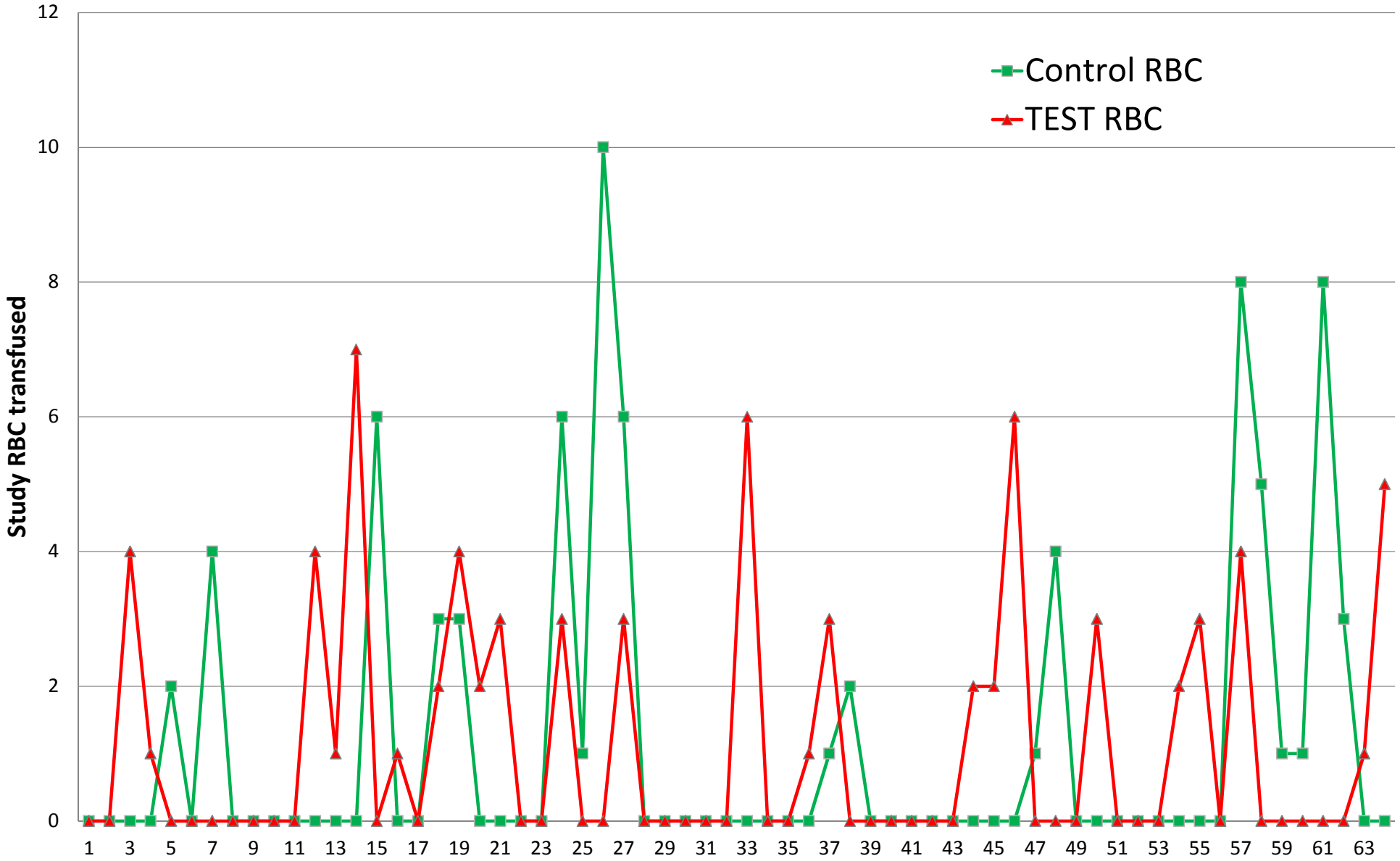
Study RBC

- Study RBC produced for the clinical trial 774
- Number of TEST RBC (S-303 treated) 402
- Number of Control RBC (untreated) 372
- RBC transfused 148
- RBC delivered (including returned RBC) 507
- Acridin Antibody Test 291
- Study RBC per patient 2.9 [1-8]
- Average RBC age at day of transfusion
  TEST RBC 18.1 days
  Control RBC 19.5 days



RBC age at transfusion

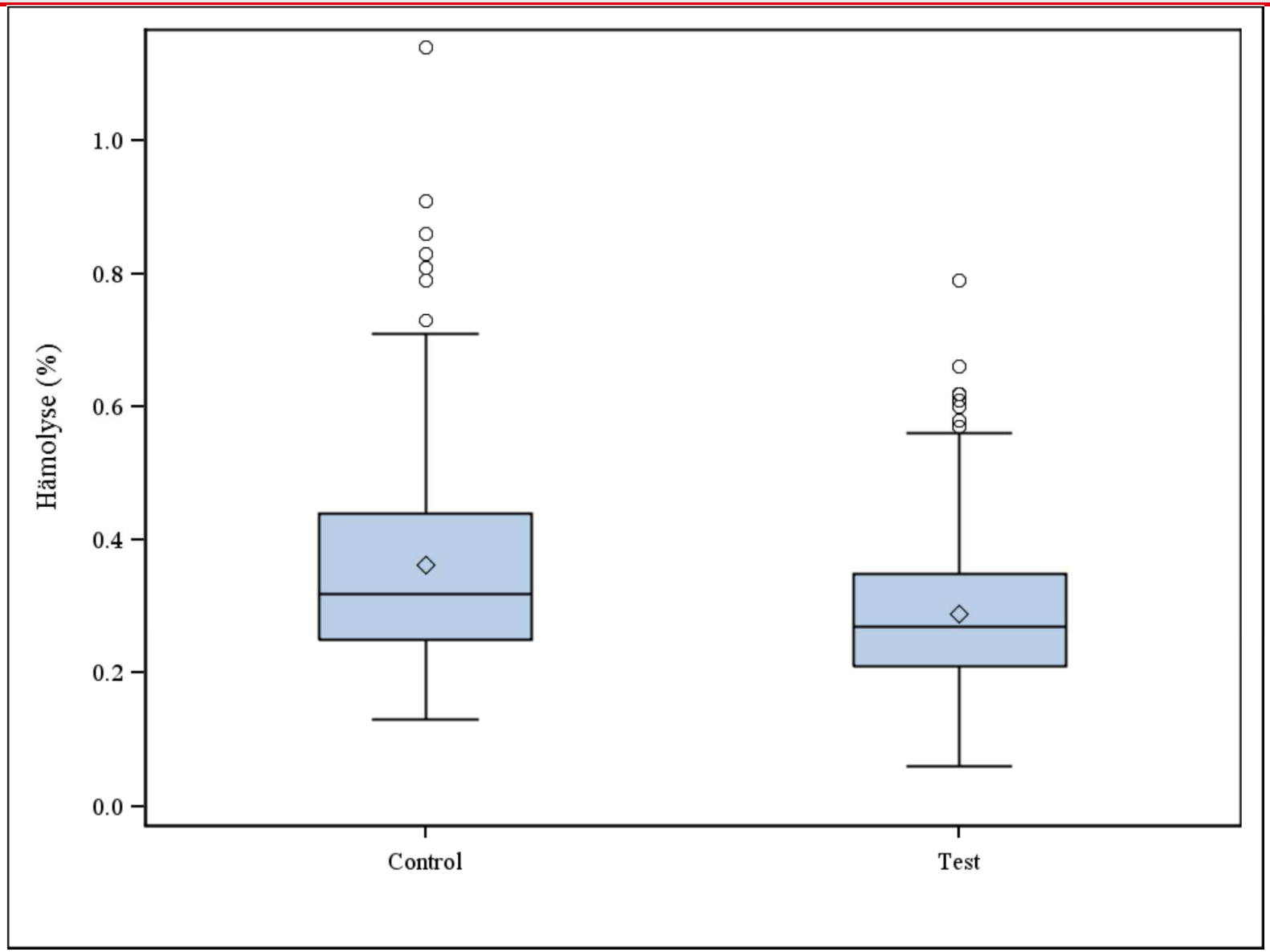
The „fever curve“ of our clinical trial – study RBC transfused per week



Study RBC QC Data 1

	Test RBC	Control RBC	P-Value
Primary Endpoint			
Post-Production Hemoglobin Content (g/unit)			
N	389	365	
Mean (SD)	53.6 (5.6)	56.3 (6.0)	0.500
Secondary Endpoints			
End-of-Storage Hemoglobin Content (g/unit)			
N	301	261	
Mean (SD)	53.1 (5.7)	55.8 (5.9)	0.691
End of Storage Hematocrit (%)			
N	301	261	
Mean (SD)	60.4 (3.2)	60.9 (3.5)	1.000
End of Storage Hemolysis (%)			
N	301	261	
Mean (SD)	0.28 (0.12)	0.35 (0.16)	0.021

Hemolysis at end of shelf life TEST versus Control RBC

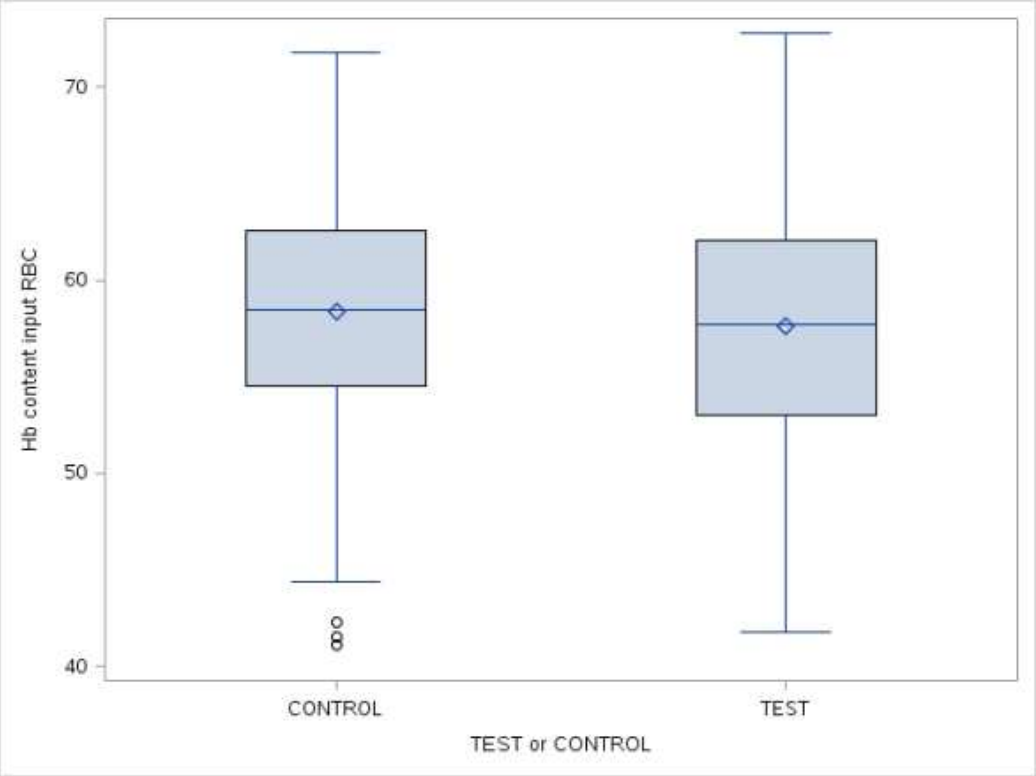


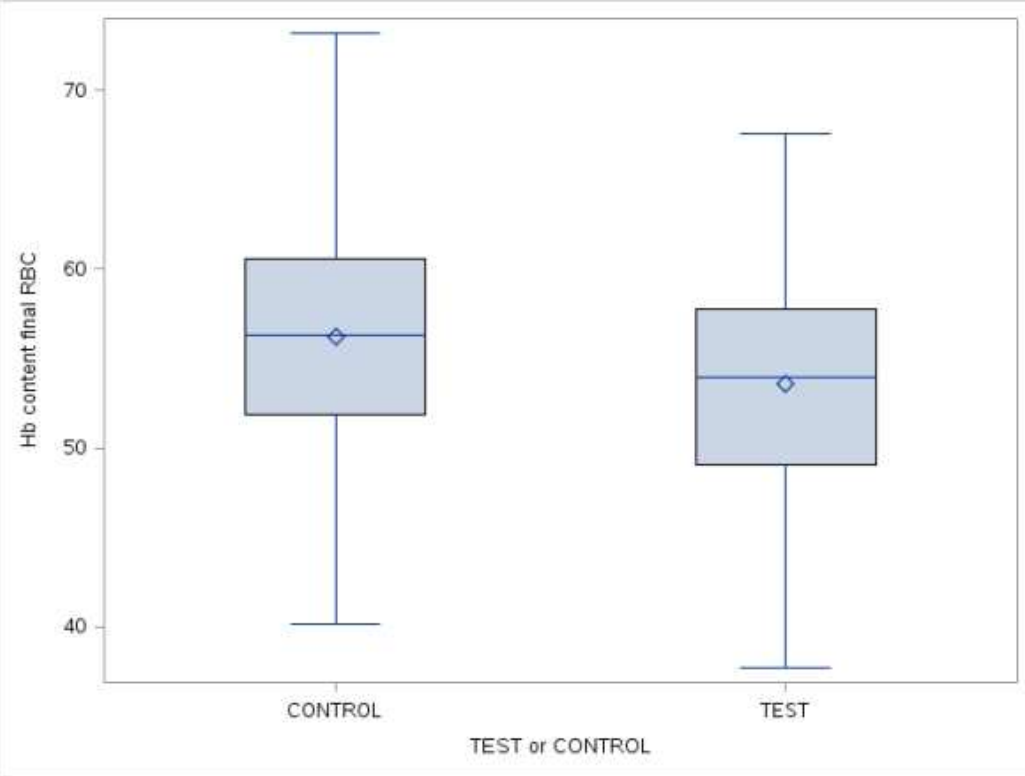
Study RBC QC Data 2

Quality Parameter	Timepoint	Control RBC	Test RBC
Hematocrit (%)	PP	57.3±2.9 (n=367)	57.4±2.0 (n=389)
MCV [fL]	PP	87.5±5.0 (n=367)	85.7±4.8 [c] (n=389)
	EOS	93.6±6.4 (n=225)	91.3±6.6 [c] (n=263)
MCHC [g/dl]	PP	33.6±1.1 (n=367)	34.2±1.2 [c] (n=389)
	EOS	31.4±1.0 (n=225)	32.3±1.4 [c] (n=263)
pH	EOS	6.4±0.1 (n=256)	6.3±0.1 [c] (n=293)
Potassium [mmol/L]	EOS	41.8±3.6 (n=261)	40.6±3.3 [c] (n=243)
Glucose [mmol/L]	EOS	308.2±33.2 (n=163)	317.4±31.0 [c] (n=259)
Lactate [mmol/L]	EOS	29.1±3.3 (n=225)	20.4±2.4 [c] (n=262)
Total Protein [mmol/L]	EOS	228.8±34.7 (n=298)	68.0±25.6 [c] (n=301)

[a] PP: Post Production (Day 2)
[b] EOS: End of Storage (Day 35-38)
[c] p<0.05; p-values for the mean treatment difference (T-C) are based on a T-test with unequal variances.

Hemoglobin content – Input component versus final study RBC



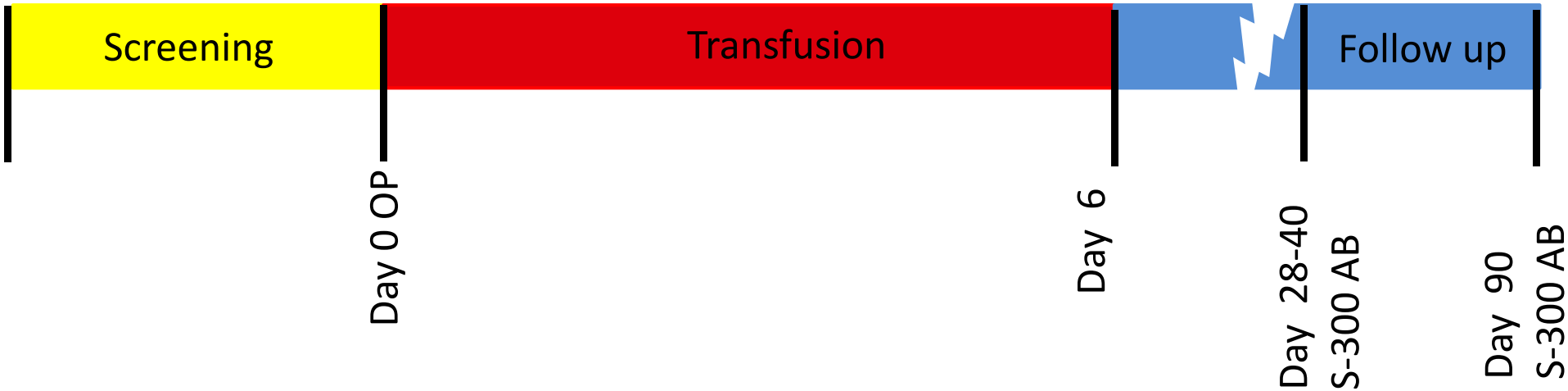


	Control	Test
Mean Hemoglobin content input RBC	58.4 (6.0)	57.6 (5.8)
Mean Hemogobin content post production	56.3 (6.0)	53.6 (5.6)

Inclusion criteria

- **Age ≥18** years, of either gender.
- Must be willing to use an **acceptable form of contraceptive** while on study (as approved by the Investigator or designee)
- Must be readily available by telephone
- EC- approved informed consent
- Must have a negative cross match to S-303 RBCs at study entry **(no pre-existing Ab specific to S-303 treated RBC)**
- Must have a blood type of either **A+ or O+**
- Patients must have a **high likelihood of receiving a transfusion** as determined by the Investigator OR a TRUST Score of ≥3 at study entry
- Must be scheduled to receive one of the following operative procedures (exceptions possible):
  - Coronary artery bypass graft only, first procedure
  - Valve repair or replacement only, first procedure
  - A combination of first time CABG and valve repair or replacement

Study flow chart



Inclusion- and
Exclusion criteria
Blood Group
Acridin Antibody Test
IAT

Patient receives study RBC
documentation of AE und SAE

Documentation of
AE and SAE

Patients transfused

- Patients enrolled: 87
- Patients transfused: 51
- Patienten with off-protocoll transfusion: 4
- No adverse reaction due to study RBC
- No mistakes during product assignment, crossmatching or RBC delivery

Transfused Patients

	Randomized patients with any study RBC exposure (n=51)		
	Test (n=25)	**Control** (n=26)	P-Value]
Baseline Variables			
Age (years)	73.9 (7.7)	74.3 (6.5)	0.861
Proportion of Females	11 (44.0%)	16 (61.5%)	0.192
Body Mass Index (kg/m^2)	27.8 (5.8)	26.4 (4.2)	0.317
Baseline Hgb (g/dL)	12.7 (0.8)	12.4 (1.2)	0.217
Overall Surgical Variables			
Overall Proportion of Bypass Pump Use	22 (88.0%)	23 (88.5%)	0.912
Overall Proportion of Aortic Cross Clamp Use	22 (88.0%)	23 (88.5%)	0.912
Overall Proportion of Cell Saver Use	13 (52.0%)	15 (57.7%)	0.781
Est Vol of Surgical Bld Loss (L)	1.57 (2.13)	1.32 (0.93)	0.63
Proportion With Surgical Complications Leading to Additional Blood Usage	1 (4.0%)	2 (7.7%)	0.631
Transfusion Variables			
Number of Study RBC Units Transfused	2.9 (1.7)	2.9 (2.0)	0.87
Age of Transfused Study RBCs (days)	18.1 (8.6)	19.6 (8.1)	0.253
Est Vol of Non-Study RBCs Transfused (L)	3.17 (4.62)	1.14 (0.64)	0.625
Proportion With Platelet Exposure	7 (28.0%)	8 (30.8%)	0.91

Development of antibodies in study patients during the clinical trial

- Antibodies against acridine were not detected in any study patient at any time during the clinical trial
- A patient in the TEST treatment group [02B-0036] developed a Jk(a) antibody without clinical signs of hemolysis
- A patient in the TEST treatment group [01B-0001] developed a Lu(a) antibody without clinical significance

Adverse Events

Overall Summary of Treatment-Emergent Adverse Events (MITT Patients)

	Valve Only		CABG Only		Valve and CABG		All Patients			
	Test (N=10)	Control (N=8)	Test (N=12)	Control (N=13)	Test (N=3)	Control (N=5)	Test (N=25)	Control (N=26)	Total (N=51)	P-Value [1]
AE by Worst Severity										
No AE	1 (10.0%)	2 (25.0%)	2 (16.7%)	3 (23.1%)	0	0	3 (12.0%)	5 (19.2%)	8 (15.7%)	0.149
Grade 1	0	0	0	0	1 (33.3%)	2 (40.0%)	1 (4.0%)	2 (7.7%)	3 (5.9%)	
Grade 2	1 (10.0%)	2 (25.0%)	4 (33.3%)	7 (53.8%)	0	0	5 (20.0%)	9 (34.6%)	14 (27.5%)	
Grade 3	4 (40.0%)	1 (12.5%)	3 (25.0%)	2 (15.4%)	0	1 (20.0%)	7 (28.0%)	4 (15.4%)	11 (21.6%)	
Grade 4	3 (30.0%)	2 (25.0%)	2 (16.7%)	1 (7.7%)	1 (33.3%)	1 (20.0%)	6 (24.0%)	4 (15.4%)	10 (19.6%)	
Grade 5	1 (10.0%)	1 (12.5%)	1 (8.3%)	0	1 (33.3%)	1 (20.0%)	3 (12.0%)	2 (7.7%)	5 (9.8%)	
Total	10 (100%)	8 (100%)	12 (100%)	13 (100%)	3 (100%)	5 (100%)	25 (100%)	26 (100%)	51 (100%)	
AE by Strongest Relation										
No AE	1 (10.0%)	2 (25.0%)	2 (16.7%)	3 (23.1%)	0	0	3 (12.0%)	5 (19.2%)	8 (15.7%)	0.314
Excluded	3 (30.0%)	2 (25.0%)	4 (33.3%)	4 (30.8%)	1 (33.3%)	2 (40.0%)	8 (32.0%)	8 (30.8%)	16 (31.4%)	
Unlikely	4 (40.0%)	4 (50.0%)	5 (41.7%)	5 (38.5%)	0	1 (20.0%)	9 (36.0%)	10 (38.5%)	19 (37.3%)	
Possible	2 (20.0%)	0	1 (8.3%)	1 (7.7%)	2 (66.7%)	2 (40.0%)	5 (20.0%)	3 (11.5%)	8 (15.7%)	
Total	10 (100%)	8 (100%)	12 (100%)	13 (100%)	3 (100%)	5 (100%)	25 (100%)	26 (100%)	51 (100%)	

Summary

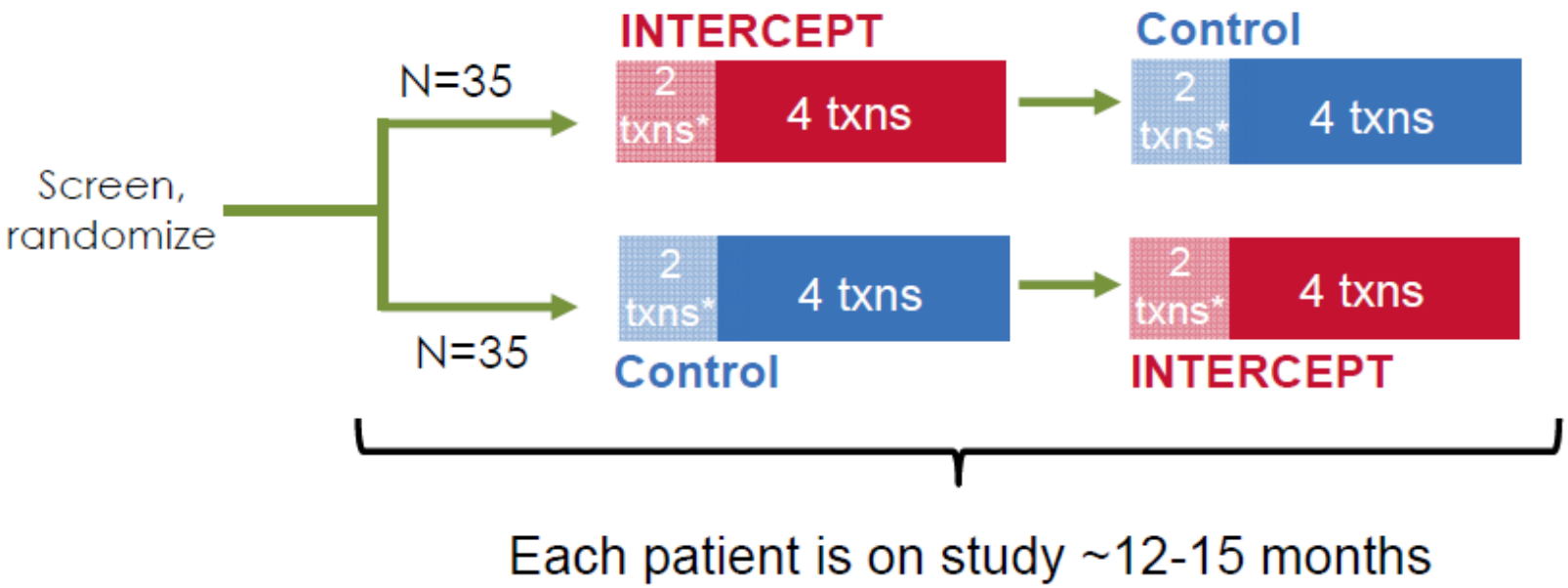
- PI RBC in this study were non-inferior compared to control RBC regarding the mean hemoglobin content post production (primary endpoint)
- S-303 treated RBC were transfused in two clinical centers without any transfusion reaction or adverse event with probable or definite relation to study medication
- Average number or study RBC transfused per patient was comparable in TEST and Control patient group (2.9 RBC per patient in both groups)
- QC data of TEST and CONTROL RBC showed promising results for S-303 treated RBC – low hemolysis rate, low total protein concentration in TEST RBC

Chronic Transfusion Study

Transfusion-dependent thalassemia major patients (n = 70)

1° efficacy endpoint = Hemoglobin usage

1° safety endpoint = Immunogenicity with repeat exposure



Whole Blood Pathogen Inactivation
Safe Blood for Africa

Anaïs ALTMEYER, Ph.D
Transfusion Swiss Red Cross

BLUTSPENDE SRK SCHWEIZ
TRANSFUSION CRS SUISSE
TRASFUSIONE CRS SVIZZERA

Members of the Scientific Advisory Board

- **Transfusion Swiss Red Cross :** Dr Rudolf SCHWABE
- **Experts:** Dr Jeffrey McCULLOUGH, Univ. of Minnesota (USA)
  Dr Chrisof GEISEN, Univ. of Frankfurt (Germany)
  Dr Jean-Pierre CAZENAVE, EFS Alsace (France)
  Dr Christian GACHET, EFS Alsace (France)
- **Cerus (Concord, USA) :** Dr Larry CORASH
  Dr Nina MUFTI
  Dr Anne NORTH – Dr Tovo DAVID
- **Research Team :** Dr Soraya AMAR EL DUSOUQUI
  Dr Anaïs ALTMEYER
  Dr Emmanuel RIGAL – Dr Andreas BUSER

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Our African partners

- Dr Claude TAYOU: University Hospital, Yaoundé – CAMEROON
- Dr Mamadou Yassongui SEKONGO: National Blood Transfusion Center, Abidjan – IVORY COAST
- Dr Ludovic ANANI: National Agency for Blood Transfusion, Cotonou – BENIN

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Current situation of blood bankers in low resource countries

- Availability of only 40% of blood products needed
- Zone of high prevalence of malaria, HCV, dengue fever and HIV
- No serological tests for HIV, HBV, HCV and syphilis for each donation
- Frequent inability to irradiate blood bags collected from family donnors
- 75% of donations are transfused as whole blood units

- **Adaptation of CERUS' INTERCEPT Technology for Red Blood Cells**

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Phases of the WB PI project

Project Planning	Phase A	Phase B	Phase C
Preparation and detailed planning	Construction and *in vitro* feasibility studies of technology transfer and pathogen inactivation (PI) in whole blood products Partial step depends on further demand of ethics Committee and HA of further needed in-depth research on the effectiveness of PI	Clinical trial in Africa : efficacy and safety of PI whole blood transfusion in treating acute anemia	Securing the sustainability of the findings and implications of the successful transfer Project completion

Monitoring and Evaluation

Measures to ensure acceptance, communication and information

Current phase: Phase A

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RESULTS : Coagulation factors

- **Primary outcome of our study:** preserve erythrocytes functions to treat acute anaemia but not coagulation disorders

- % of coagulation factors is decreasing when increasing GSH concentration

- **The compromise:** choose a GSH concentration that preserves coagulation factors ?

Coagulation Analysis (mechanical testing) at CERUS (Geneva samples)

	No treatment	S-303 only	2 mM GSH	5 mM GSH	7.5 mM GSH	10 mM GSH	20 mM GSH	Required for Hemostasis (% of normal)
PT (sec)	12.1	15.1	18.4	21.6	26.6	30	52.7	
aPTT (sec)	33.1	46.7	54.7	70.8	116	123	no clot	
Percent of Control								
FII		54.2	58.1	62.4	60.9	59.9	61.2	40
FV		65.5	74.4	77.9	78.8	81.1	68.7	10-15
FVII		31.5	49.8	41.1	42.6	37.5	22.4	5-10
FVIII		36.1	66.9	56.5	48.2	61.8	44.1	10-40
FIX		34.9	43.9	36.0	26.0	19.4	9.4	10-40
FX		41.1	32.4	19.2	11.9	9.2	3.9	10-15
FXI		65.3	62.6	48.8	36.5	31.9	11.2	20-30
FIB		81.1	82.2	83.2	77.3	76.4	74.3	30

Conclusion: Individual coagulation factor activity showed similar results as seen before, reduced factor activity with increased GSH concentration. Some individual factors are more affected than others.

Conclusion of the second SAB meeting

Conservation of RBC general characteristics confirmed across all GSH concentrations

Effect of GSH on coagulation factor activity confirmed with mechanical assay of coagulation activity; some attributes of the ROTEM assay also effected

Little preservation of platelet function across all GSH concentrations
Recommend SAB to consider GSH concentration less than 20 mM and less than 10 mM

Effect of *Plasmodium* inactivation in whole blood on the incidence of blood transfusion-transmitted malaria in endemic regions: the African Investigation of the Mirasol System (AIMS) randomised controlled trial

Lancet 2016; 387: 1753–61

Jean-Pierre Allain, Alex K Owusu-Ofori, Sonny Michael Assennato, Susanne Marschner, Raymond P Goodrich, Shirley Owusu-Ofori

Summary

Background Transfusion-transmitted malaria is a frequent but neglected adverse event in Ghana. We did a randomised controlled clinical trial to assess the efficacy and safety of a whole blood pathogen reduction technology at preventing transfusion transmission of *Plasmodium* spp parasites.

Findings Between March 12, 2014, and Nov 7, 2014, 227 patients were enrolled into the study, one of whom was subsequently excluded because she did not meet the inclusion criteria. Of the 226 randomised patients, 113 were allocated to receive treated whole blood and 113 to receive standard untreated whole blood. 223 patients (111 treated and 112 untreated) received study-related transfusions, whereas three patients (two treated and one untreated) did not. 214 patients (107 treated and 107 untreated) completed the protocol as planned and comprised the per-protocol population. Overall, 65 non-parasitaemic patients (28 treated and 37 untreated) were exposed to parasitaemic blood. The incidence of transfusion-transmitted malaria was significantly lower for the pathogen-reduced (treated) patients (1 [4%] of 28 patients) than the untreated group (8 [22%] of 37 patients) in this population (p=0·039). Overall, 92 (41%) of 223 patients reported 145 treatment-related emergent adverse events during the conduct of the study, with a similar incidence of adverse events between groups receiving untreated or treated whole blood. No transfusion-related deaths occurred in the trial.

Interpretation Treatment of whole blood with the Mirasol pathogen reduction system for whole blood reduced the incidence of transfusion-transmitted malaria. The primary endpoint of the study was achieved in the population of non-parasitaemic patients receiving parasitaemic whole blood. The safety profile and clinical outcomes were similar across the two treatment groups.

A SLIDE KINDLY PROVIDED BY WIM DE KORT:

THE FUTURE OF PATHOGEN REDUCTION

"Prediction is very difficult, especially, when it concerns the future."

Danish Proverb

Alliance of Blood Operators

Paris 2014

Special thanks to …

Dr. Veronika Brixner
Dr. Christof Geisen
H.-U. Pfeiffer
Johannes Leibacher
Sarah Dombos
Iuliia Weber
Tanja Wotapek
Diana Hedderich

Dr. Arndt-Holger Kiessling
Sonja Friese
Pamela Ilioska

Dr. Katharina Madlener
Dr. Larissa Wohlgemuth
Gülten Baran

Prof. Reinhard Henschler
Prof. Erhard Seifried

… and to all of you for your kind attention!